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RESEARCH ARTICLE

A Hydroponic Approach to Evaluate Responses of Kodomillet (*Paspalum scrobiculatum*) germplasm against NaCl stress

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Abstract

Kodo millet is one of the small grain cereals with an ability to tolerate the biotic and abiotic stresses. Soil salinity is one of the major stresses especially in arid and semi-arid regions leads to reduced yields. In our work we studied the physiological and biochemical responses of six varieties of kodo millet germplasm (IPS 145, IPS 610, IPS 351, IC 382888, IPS 583 and IC 426676) against NaCl salinity through hydroponic experiment. Germinated seeds were grown in beakers supplemented with Hoagland nutrient solution containing NaCl (0, 50, 100, 150 and 200 mM) for about 120 hours and the data was collected for every 18 hours interval on root length, shoot length, RWC, proline, CAT and SOD. Among all the varieties IC 426676 and IPS 583 showed highest root, shoot length and RWC, whereas the lowest were observed in IPS, 145, 610, 351 and IC 382888 genotypes. With an increase in salt levels from 0 to 200 mM, the activity of proline increased when compared to their respective controls in all test varieties. Superoxide dismutase (SOD) activity found to be increased and the activity of CAT decreased among all the germplasms with an increase in NaCl concentration. In conclusion, IC 426676 and IPS 583 were performed well against different NaCl stress levels and can be used further under arid and semi-arid conditions.

Introduction

Soil salinity is a great problem in many areas of the world where it causes significant reductions of crop yield (Tavakkoli *et al.*, 2011; Hajiboland *et al.*, 2009). This process is characterized as the main factor of world soil degradation (Grieve and Maas, 1984) that affects the yields around 60 to 80 million ha (Grieve and Shannon, 2001). Each year, more and more land becomes non-productive owing to salt accumulation. At least 25% of currently cultivated land throughout the world suffers from excess salinity principally from sodium chloride (NaCl). Crops grown in salt affected soils may suffer from osmotic stress, ion toxicity, and mineral deficiency leading to reduced growth and productivity (Netondo *et al*., 2004). Cellular dehydration is a general consequence of osmotic stresses, including water deficit at high salinity levels (Incharoensakdi *et al*., 1986; Robinson and Jones, 1986).

The quantitative nature of stress tolerance and the problems associated with developing appropriate and replicable testing environments make it difficult to distinguish stress-tolerant lines from sensitive lines. One approach to a better understanding of plant stress tolerance is to identify the morphological characteristics that are proposed to contribute to stress tolerance and to determine their relative importance (Munns, 2002).

One of the most common stress responses in higher plants is over production of some various compatible organic solutes (Serraj and Sinclar, 2002). These solutes are most commonly carbohydrates like sugars, amino acids

and proteins that acts as osmolytes. Proline is one of them that known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses (Kishor *et al.,* 2005). In a number of studies a positive correlation between the accumulation of these osmolytes and stress tolerance has been reported (Yamada *et al.,* 2003). Fozouni *et al.* (2012) (Yamada *et al.,* 2003; Fozouni *et al.* (2012) have demonstrated the same responses in hydroponically grown cultivars.

Plant cells have evolved a complex defensive mechanism in response to abiotic and biotic stresses, which is composed of low molecular mass antioxidants (glutathione, ascorbate and carotenoids) as well as ROS scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) (Bhardwaj *et al.,* 2007).

Growing plants in hydroponic solution with an addition of salinizing salts is an easy technique that rigorously controls the root environment for evaluation of the response of the plants to salinity (Feigin *et al.,* 1987; 1992 and Shibli, 1993). With this technique most of the complexities and interferences induced by soil and environmental factors are avoided and better control of the experiment is achieved (Meyer *et al.,* 1989). Basing on the above conclusions the present study was taken up with an aim to evaluate morphological and biochemical responses of kodo millet to different salt stress levels in selected germplasm of Kodomillet through hydroponic system.

Material and Methods

Plant material and salinity treatment

Kodo millet (*Paspalum scrobiculatum* L.) germplasm such as IPS 145, IPS 610, IPS 351, IC 382888, IPS 583 and IC 426676) were obtained from ICRISAT and NBPGR, India. The collected seeds were surface sterilized and were placed on a thin bit of moist sponge kept in 250 ml beakers containing half strength modified Hoagland solution (Hershy and Merritt, 1986). The solution in beakers was aerated with an aerator for 5 minutes both in dusk and dawn. Seeds were left to germinate on half strength Hoagland's solution without stress up to 2 mm emergence. Later seedlings were treated with NaCl having concentrations of 0, 50 mM, 100 mM, 150 mM and 200 mM upto 120 hours. Data was taken at every 18 hours after the treatment. After 120 hrs of stress final data was recorded in treated and controlled beakers.

Root length

The length of all the roots was measured with the help of a measuring scale and expressed in centimetres.

Shoot length

Plant height was recorded from the ground level to the growing tip of the main shoot and calculated using scale and expressed in centimetres.

Relative Water Content (RWC)

Relative water content was estimated according Fletcher *et al.,* (1988) on the final day of the experiment and calculated by the formula given by Kramer (1983)

$$RWC = \frac{\text{Fresh weight - dry weight}}{\text{Turgid weight - dry weight}} \times 100$$

Proline activity

The extraction and estimation of Free Proline was done in the seedlings according to Bates *et al.* (1973). Fresh leaf material of (0.5 g) was homogenized in 10 ml of 3% sulfosalicylic acid and the homogenate was filtered. Two milliliters of filtrate was treated with 2 ml ninhydrin reagent (1.25 mg ninhydrin in 30 ml of glacial acetic acid

and 20 ml of 6 molar H_3PO_4) and incubated at 95°C for 1 h. The reaction was terminated placing in an ice bath. The reaction mixture was vigorously mixed with 4 ml toluene. After warming at 25°C, absorbance of the colored solutions was read at 520 nm.

Catalase activity (CAT)

Germinating seeds were taken and seed coat was removed, 1 g of this material was macerated into thin paste using pH 7 Phosphate buffer and the enzyme extract was filtered through muslin cloth. Two milliliters of the enzyme extract taken into 50 ml clear conical flask and to this 1 ml of 0.45 molar H_2O_2 was added and the set up was kept for 5 min incubation and enzyme activity was stopped by adding 1 ml of 12 % H_2SO_4. This extract was titrated against 0.05 N of $KMnO_4$ taken in a burette, appearance of pink color and remains constant for about 30 seconds considered as the end point. The amount of H_2O_2 destroyed by catalase is calculated by the formula, the enzyme activity was expressed as enzyme units per gm leaf material. One unit of catalase is defined as that amount of enzyme, which breaks down /µmol/ of H_2O_2 / min.

$$\text{Catalase activity} = \frac{25 \times 0.85}{2} \times \frac{V}{W}$$

Where W= Weight of material used
V= Volume of $KMNO_4$ utilized (Blank sample value)

Superoxide dismutase activity (SOD)

Leaf samples of 500 mg were homogenized in ice cold 50 mM potassium phosphate buffer (pH 7.8) with pre-chilled pestle and mortar. Each homogenate was transferred to centrifuge tubes and was centrifuged at 4°C in cooling micro centrifuge (Eppendorf – 5415 R) at 10, 000 rpm. The supernatant was used for enzyme activity assay (E.Esfandiari *et al.*, 2007) within 12 h of extraction.

SOD activity was estimated by recording the decrease in absorbance of superoxide nitro blue tetrazolium complex by the enzyme (Gupta *et al.*, 1993). A reaction cocktail of 33 ml was prepared by mixing the reagents in the following ratio (60 µl-50 mM Phosphate buffer; 390 µl-13 mM Methionine; 0.6 µl- 02 µM Riboflavin; 60 µl-0.1 mM EDTA; 300 µl-75 mM NBT; 50 µl-Enzyme extract).

A blank was set without enzyme and NBT to calibrate the spectrophotometer. Another control was set having NBT but no enzyme as reference control. All the tubes were exposed to 400 W bulbs (4 * 100 W bulbs) for 15 min. The absorbance was measured at 560 nm immediately and calculated the percentage inhibition of the reaction between riboflavin and NBT in the presence of methionine which is taken as 1 unit of SOD activity. The enzyme activity was expressed as units/mg of protein.

Result and Discussion

Hydroponics is a mechanism of growing plants with their roots submerged in aerated water and is therefore the best way to separate the effects of aeration, effects due to supply of water and nutrients and those due to mechanical resistance because plant roots need both air and water to survive which are provided in this system. Plants absorb virtually all of the essential elements through their roots since the macro and micronutrients are dissolved in the water to supply the plants (David, 2004). During growth in hydroponics system accumulation of solutes, either actively or passively, is an important adaptation mechanism for plants in response to osmotic stress

The root length was ranged from 3.49 to 1.80. Among all the varieties the highest root length was maintained by IC 426676 and it was followed by IPS 583. The lowest was observed in IPS 145. At 50, 100, 150, and 200 mM the root length was found to be low when compared with controls. The maintenance of root length is very important during the times of salinity stress. Among all the germplasm, the shoot length in control seedlings was ranged from 1.80 cm (IPS 145) to 8.70 cm (IC 426676). Accession IC 426676 and IPS 145, showed a maximum (8.70 cm) and minimum (1.80 cm) shoot length, respectively. At 150 mM, 200 mM treatment very poor shoot length observed in IPS 145 and IPS 610 (Fig 1). Shoot length was significant in IC 382888, IPS 583 and IC 426676 up to 150 mM concentration and it is significant up to 100 mM in other varieties IPS 145, IC 382888 and IPS 351 (Fig 2).

The seedlings growth is normally limited with an increase in NaCl concentration (Sreenivasulu *et al.,* 2000). In our study, with increasing salinity levels, the root and shoot lengths were found to be reduced. The cultivars IPS 145 and IPS 610 reported less root length may be because of damaged cells and meristamatic zone due to ion toxicity. In case of shoot also reduced growth was observed which is in support with the above statement. In spite of these adverse conditions IC 426676 and IPS 583 recorded good shoot length even at 200 mM concentration.

The relative water content was showed significant variations among the treatments. The accessions IC 426676 and the IPS 583 followed by IC 382888 maintained the high rates of RWC. When compared to the above genotypes the accessions IPS 145, IPS 610 and IPS 351 recorded less relative water content. In controls and 50 mM concentrations, the test varieties reported significant RWC in all varieties. But the varieties IPS 145, IPS 610 and IC 426676 found to be significant even at 100 mM concentration of NaCl (Fig 3).

As the salinity stress increases the RWC decrease in all the test genotypes. This is may be due to reduction in water supply to the cells by increasing in Na^+ ions in cytoplasm which compete with K^+ ions results in decrease in osmotic potential in cell cytoplasm of all the cultivars. The above results are in support with Murillo *et al.,* (2002) and Szigeti, (1991).

The proline activity was increased comparatively among all the varieties with increase in NaCl concentration. Proline plays an adaptive role in mediating osmotic adjustment and protecting the sub-cellular structures in stressed plants (Voetberg and Sharp, 1991). Delavney and Verma (2003) indicated that proline is more efficient to protect cells against stress comparison with other conventional osmolite compatible such as sugars and sugar alcohols. Also, proline shows an indirect protective function due to its antioxidant properties in addition to the direct effect to stabilize the macro-molecules and their hydration layers (Bohnert *et al.,* 2004). The highest proline activity was observed in accession IC 426676 and IPS 583 in all the treatments, whereas the lowest activity was exhibited in IPS 145 and IPS 610 (Fig 4). The activity of proline was significant in all the varieties at 150 mM and 200 mM, NaCl concentration, this may be due to effective osmoregulatory mechanism in kodomillet.

The increased proline activity with increase in osmotic stress was also observed in citrus cultivars (Ghotb Abadi *et al.,* 2010), in grape vine (Fozouni *et al.* 2012; Hamed *et al.,* 2013). In many plants a positive correlation between the accumulation of proline and stress tolerance has been found.

As the concentration of salinity increases the available sugars automatically decreases. Because Salt stresses could cause oxidative damage. Therefore, plant cells need different mechanisms, which enable the detoxification of excess reactive oxygen species (ROS) and keep the balance of the formation and removal ROS. The decreased activities of CAT detected in this study are presumed to enhance cellular damage and limits the plant's antioxidative capacity to defend stress. The lowest decrease in catalase levels was found in the variety IC 426676 in all the treatments. The highest reduction in the catalase was found in the variety IPS 145 and IPS 610 in all the treatments. The depletion in catalase availability was found to be significant up to 100 mM in all the varieties and it was only upto 50 mM in the accessions IPS 583 and IC 426676 (Fig 5).

SOD is one of the major antioxidative enzyme present in all aerobic organisms and mostly in subcellular components which generate activated oxygen (Sajjad *et al.,* 2012). The present study reported that SOD activity increased with increasing NaCl concentration (Fig 6). This increased SOD activity is useful to regulate the plant defensive mechanism (Jaleel *et al.,* 2007a) under abiotic stress conditions. Moreover superoxide dismutase would play a crucial protective role under anoxic conditions, prevailed due to salinity stress. The accessions IC426676 and IPS 583 produced more SOD among all the cultivars in all the NaCl treatments. The present study support the hypothesis that antioxidative enzymes play a central protective role in the detoxification of O_2 and H_2O_2 by scavenging process with coordination of SOD (Liang *et al.,* 2003, Badawi *et al.,* 2004).

Conclusion

Use of hydroponic methods to screen the salt tolerant cultivars found to be successful and dependable especially in case of neglected minor millet such as Kodomillet. This is proved by the present study. In the present study accessions IC 426676 and IPS 583 showed their potency in survivability and growth when treated with different concentrations of NaCl. These cultivars can be used further to examine their tolerance through pot culture and field trials in order to suggest them as potent lines to with stand to the high saline soils predominantly sodium chloride stress.

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Fig 1. Effect of NaCl salinity on Root length

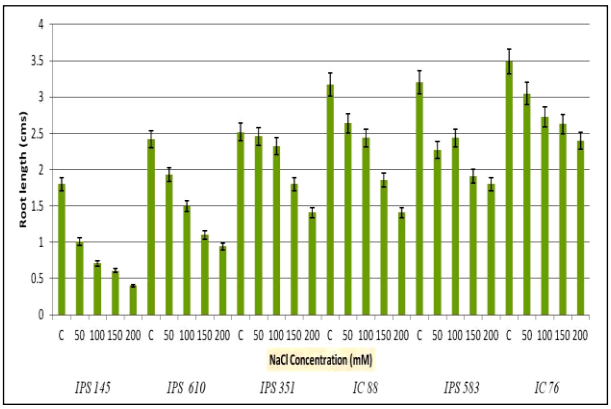

Fig 2. Effect of NaCl Salinity on Shoot length

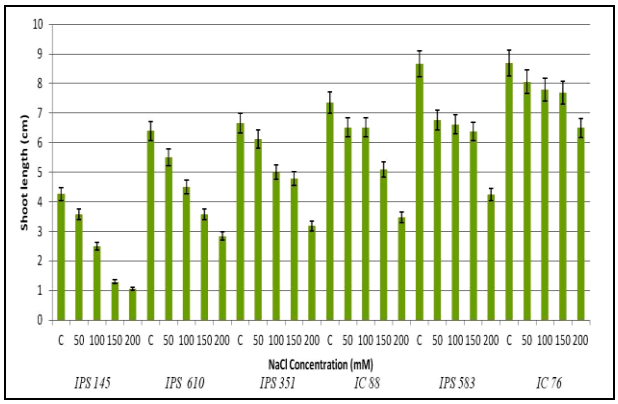

Fig 3. Effect of NaCl salinity on Relative Water Content

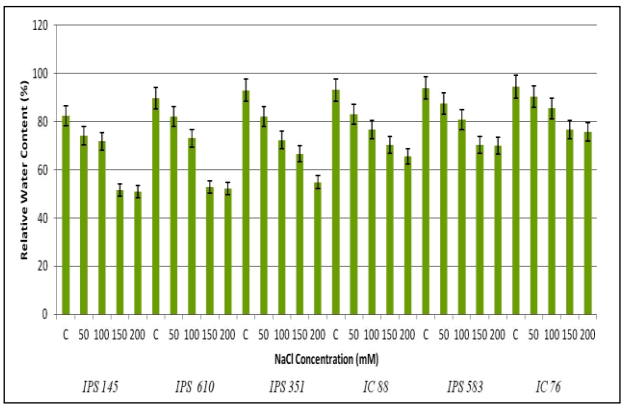

Fig 4. Activity of Proline with increased NaCl concentration

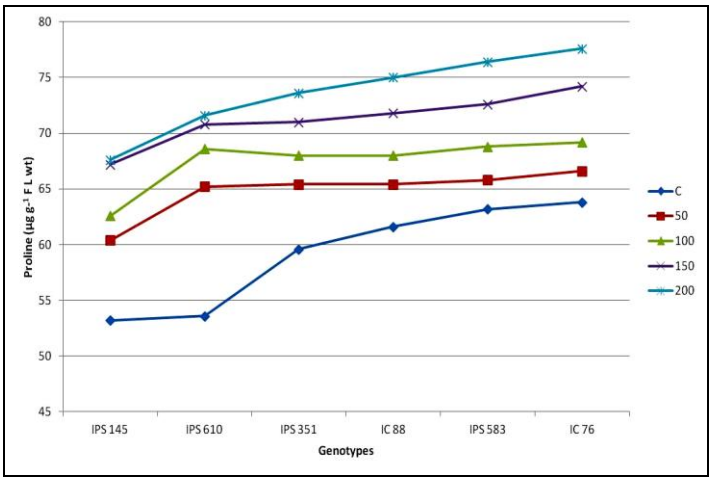

Fig 5. Catalase activity on kodo millet seedlings with increased NaCl concentration

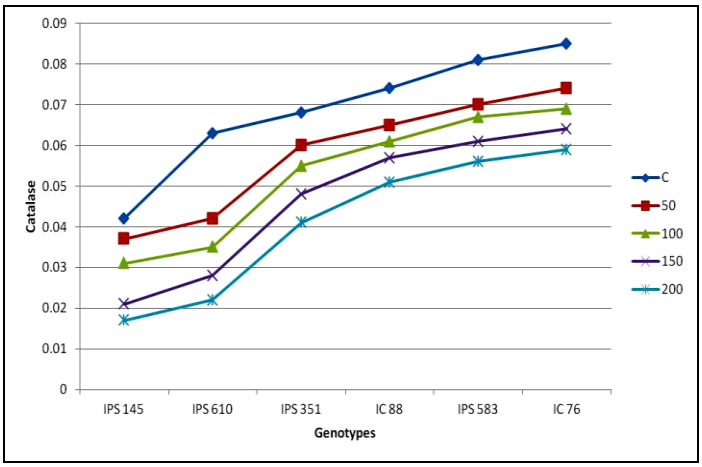

Fig 6. Activity of SOD against increased NaCl concentration